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Coenocystis inconstans, a New Species of Bark-Inhabiting Green Algae (Chlorophyceae, Chlorophyta)

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An alga with mucilaginous matrix around the cell wall was isolated from the bark of *Ulmus campestris* var. *vulgaris* collected from Christchurch, New Zealand. The cells when young are ellipsoidal to narrowly ellipsoidal in shape, and ovoid or spheroidal when mature. The chloroplast of young cells is trough-like or cup-shaped with smooth margin, while that of mature cells is often divided into a few lobes. The unbranched thylakoid lamellae penetrate the pyrenoid in almost parallel arrangement. Many starch grains are often located around the pyrenoid. The cells are capable of producing 2–4–(8) autospores. These characteristic features agree fundamentally with those of the genus *Coenocystis* Koršikov, the family Radiococcaceae, but any described species of this genus do not share the gross morphology with our alga. The alga is, therefore, recognized as a new species named *Coenocystis inconstans* Hanagata & Chihara.

Key words: Chlorophyceae, *Coenocystis*, new species

Introduction

One of the significant features which characterize the family Radiococcaceae Fott et Komárek is the presence of mucilage around cells. Komárek and Fott (1983) divided the family into four subfamilies, mainly on the basis of the features of the surrounding mucilage: the Radiococcoideae, the Disporoideae, the Dictyochlorelloideae and the Palmodictyoideae.

The genus *Coenocystis* Koršikov (1953), with *C. planctonia* Koršikov as the type, is a member of the Radiococcoideae. Komárek and Fott (1983) listed seven species and two varieties in this genus, most of which inhabit freshwater. In the course of our studies of bark-inhabiting green algae, many specimens were collected and their taxonomy has been studied by light and electron microscopy (Hanagata et al. 1996a, 1996b, 1997). We encountered an alga with a mucilaginous matrix around the cells among the bark samples collected from New Zealand and the alga was recognized as a new species of *Coenocystis* on the basis of morphological features. In this paper the description of the new species, *Coenocystis inconstans* Hanagata & Chihara, and its phylogenetic position inferred from 18S rDNA sequence data are presented.

Materials and Methods

Coenocystis inconstans was isolated from the bark of *Ulmus campestris* var. *vulgaris* collected by one of us, M. Chihara from Christchurch, New Zealand, on 31 December, 1995. The alga examined in this study was cultured in Bold's basal medium (Bold 1949). The methods of obtaining and growing the unialgal culture, and the procedure of transmission electron microscopy were obtained in a previous paper (Hanagata et al. 1996a). The strain described here is maintained in the Environmental Biotechnology Laboratory, Research Center for Advanced Science and Technology at the University of Tokyo.

Total DNA was extracted in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0) using a mortar and pestle in the presence of glass microfibre filter (Whatman GF/B). The polymerase chain reaction (PCR) protocols (Saiki et al. 1988) was followed for the amplification of 18S rDNA region. PCR amplification was conducted with 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The PCR product was directly sequenced using a DNA autosequencer ABI 377 (Applied Biosystems, Foster City, CA, USA) with the dye termination method. Primers used were reported by Nakayama et al. (1996). Accession number of *Coenocystis inconstans* is AB017435.

The determined sequence was manually aligned with other previously known ones. The sequences included in this study are as follows (accession numbers): *Chlorella ellipsoidea* Gerneck (X63520), *Chlorella kessleri* Fott et Nováková SAG 211-11g (X56105), *Chlorella mirabilis* Andreeva (X74000), *Chlorella saccharophila* (Krüger) Migula (X63505 and X73991), *Chlorella sorokiniana* Shihira & Krauss SAG 211-8k (X62441), *Chlorella vulgaris* Beijerinck SAG 211-11b (X13688), *Choricystis minor* (Skuja) Fott (X89012), *Dictyochloropsis reticulata* Tschermak-Woess (Z47207), *Fusochloris perforata* (Lee & Bold) Floyd, S. Watanabe & Deason (M62999 as *Characium performatum*), *Gloeotilopsis planctonia* Iyenger & Philipose (Z28970), *Microthamnion kuetzinganum* Nägeli (Z28974), *Mychonastes zofingiensis* (Dönz) Kalina & Punčochářová (X73996 as *Chlorella zofingiensis*), *Myrmecia biatorellae* Boye-Petersen (Z28971), *Nanochlorum eucaryotum* Wilhelm et al. (X06425), *Scenedesmus fuscus* (Shihira & Krauss) Hegewald (X73995 as *Chlorella fusca*), *Tetraselmis striata* Butcher (X70802), and *Trebouxia impressa* Ahmadjian (Z21551). The data set excluded the ambiguously aligned regions and was 1754 base pairs in total length.

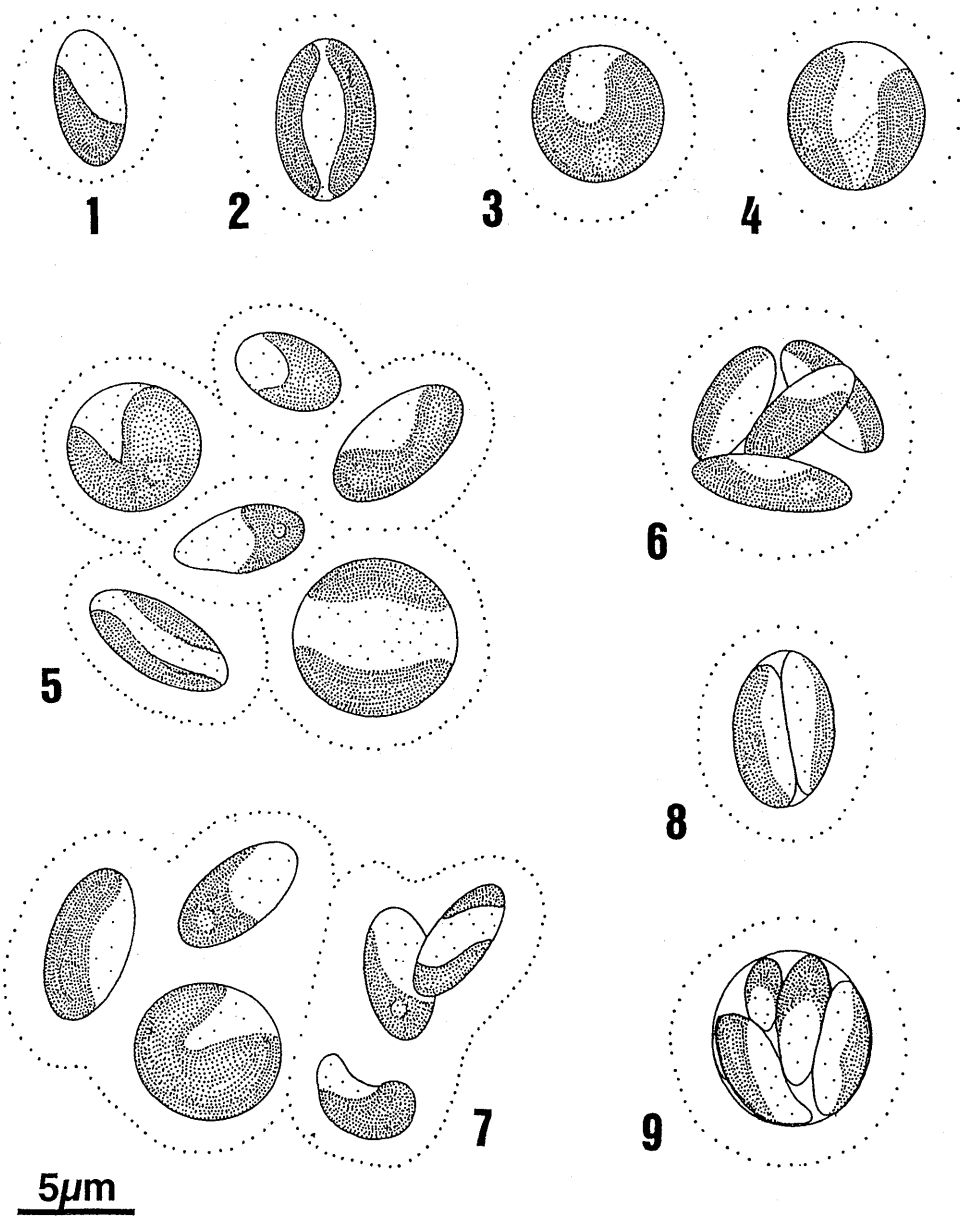
Sequences were analyzed with both the maximum parsimony and the distance matrix methods. The maximum parsimony analysis was implemented with the PAUP computer program (version 3.1.1; Swofford 1993) using a heuristic search under the unweighted scheme. A random addition of sequence with 10 replicates and a branch-swapping algorithm (TBR) was used. For the distance matrix method, Kimura's two-parameter method (Kimura 1980) was used for the calculation of the distance matrix and neighbor-joining method (Saitou and Nei 1987) to construct the tree. These procedures were conducted with CLUSTAL W (Thompson et al. 1994) and PHYLIP (Felsenstein 1993). Supporting for internal branchs in both the trees constructed with the maximum parsimony and neighbor-joining methods were estimated by the bootstrap analysis (Felsenstein 1985).

Description

Coenocystis inconstans Hanagata & Chihara, sp. nov.

Alga unicelluaris, solitaria vel colonialis in matrice gelatinosa. Cellulae juveniles ellipsoideae vel anguste ellipsoideae, rectae

vel curvatae, 2.5–4.0 × 3.5–7.5 μm. Cellulae maturae ellipsoideae vel ovatae, 3.5–6.5 × 5.0–9.5 μm, vel sphaericae, 3.5–8.0 μm in diametro. Chloroplastus unus, parietalis, cupulatus, saepe margine grandi lobus. Pyrenoides singla, granis amylaceis numerosis circumcincta. Matrix pyrenoidis per lamellas parallelas invasa. Reproductio asexualis per 2–4–(8) autosporas effecta. Autosporae ellipsoideae vel anguste ellipsoideae, 2.0–4.0 × 3.0–6.0 μm. Substantia carotenoide secundaria carens.



Figs. 1–9. *Coenocystis inconstans*, sp. nov. 1. Young vegetative cell. 2–4. Mature vegetative cells. 5. Aggregation of cells surrounded by mucilaginous substance. 6, 7. Colony embedded in common mucilaginous matrix. 8, 9. Autospores in parent cells.

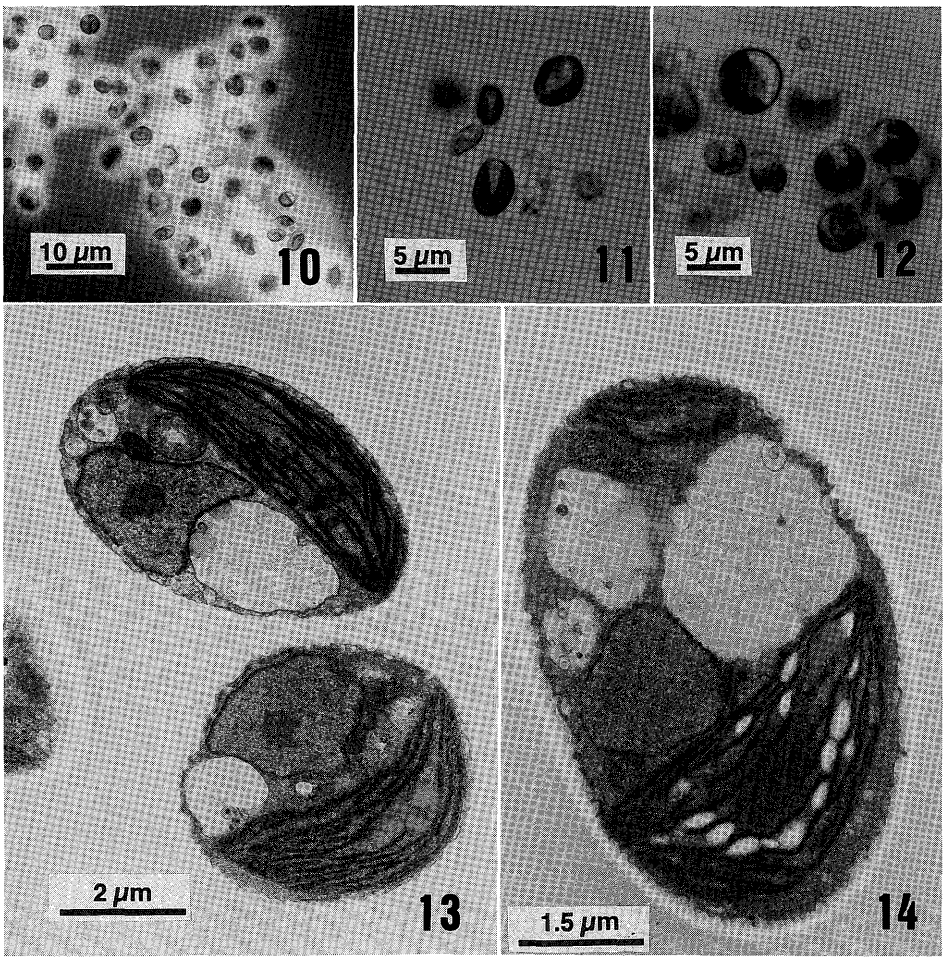
Holotype: Figure 14.

Type locality: Christchurch, New Zealand.

Habitat: Specimens examined were isolated from the samples attached on the bark of *Ulmus campestris* var. *vulgaris*.

Type culture: Christchurch, New Zealand, 31 December 1995. Strain K4-3 is deposited in the Culture Collection of Environmental Biotechnology Laboratory at Research Center for Advanced Science and Technology, The University of Tokyo.

The alga is unicellular and solitary or in colony embedded in a common gelatinous matrix. The cells are surrounded by a mucilaginous substance, whose matrix is not stratified. Young cells are ellipsoidal or narrowly ellipsoidal, sometimes ovoidal and curved, 2.5–4.0 × 3.5–7.5 μm in size, with rounded ends. Mature cells are ellipsoidal to ovoid, 3.5–6.5 × 5.0–9.5 μm in size, or spheroidal, 3.5–8.0 μm in diameter. The cell wall is composed of a single layer, 0.04–0.12 μm thick and smooth on the sur-



Figs. 10–14. *Coenocystis inconstans* sp. nov. (10–12. Photomicrographs. 13, 14. Transmission electron micrographs). 10. India ink preparation to show the presence of mucilaginous substance around cells. 11. Mature ellipsoidal cells. 12. Mature spheroidal cells. 13. Young vegetative cells. 14. Mature vegetative cell.

face. The chloroplast is parietal and cup-shaped, often with deeply divided lobes. The chloroplast consists of thylakoid lamellae that are arranged in several almost parallel rows. Several electron-dense granules, called plastoglobuli, are usually present among the thylakoid lamellae. A pyrenoid is present, but hardly distinguished from the chloroplast stroma. The pyrenoid is penetrated by unbranched thylakoid lamellae in almost parallel arrangement. Several starch grains are present among the thylakoid lamellae and also around the pyrenoid matrix, but starch accumulation is usually poor. Each cell has a single nucleus at the center, which contains a nucleolus. Reproduction takes place by formation of 2–4–(8) autospores. The autospores are ellipsoidal to narrowly ellipsoidal, 2.0–4.0×3.0–6.0 μm in size. The empty parent cell wall was not observed.

Phylogenetic position

Maximum parsimony analysis of the 18S rDNA sequences resulted in the single parsimonious tree (Fig. 15A). The length of the most parsimonious tree was 647 steps with a consistency index of 0.658. The tree constructed with neighbor-joining analysis is shown in Fig. 15B. In both trees *Coenocystis inconstans* is placed in the Trebouxiophyceae and formed a monophyletic relationship with *Chlorella ellipsoidea* and *Chlorella mirabilis*, although the bootstrap value supporting the monophyly is relative low (68 % in the maxium parsimony and 72 % in neighbor-joining analysis).

Discussion

Our alga has a mucilaginous matrix

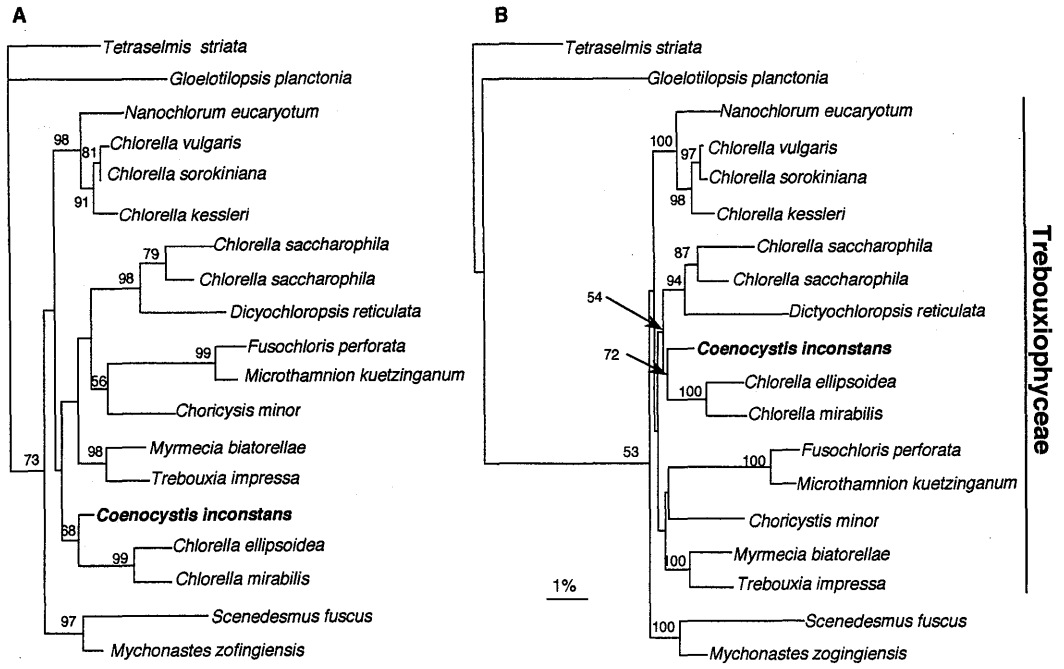


Fig. 15. Phylogenetic trees inferred from 18S rDNA sequences. A total 1754 nucleotides were considered. The numbers on the branches indicate bootstrap values (1000 replicates) larger than 50 %. A. Single most parsimonious tree generated by a heuristic searches in PAUP computer program. B. A distance tree constructed with the neighbor-joining method using CLUSTAL W computer program.

around cells, and asexually reproduces by means of autospores. These features show that our alga belongs to the subfamily Radiococcoideae, the family Radiococcaceae. This subfamily includes eight genera: *Schizochlamydella* Koršikov, *Coenochloris* Koršikov, *Thorakochloris* Pascher, *Radiococcus* Schmidle, *Eutetramorus* Walton, *Coenocystis* Koršikov, *Coccomyxa* Schmidle and *Gloeocystis* Nägeli. According to Komárek and Fott (1983), these genera are distinguishable from one another by the combination of such characters as the presence or absence of stratification of the mucilaginous sheath, the shape of cells, the arrangement of autospores, and the shape and location of the empty parent cell wall. The subfamily Radiococcoideae can be divided into two groups on the basis of the presence or absence of empty cell wall after the liberation of autospores: in one group, to which the former five genera belong, the empty cell wall remains, while in another, to which the latter three belong, it disappears. In our alga the empty parent cell wall is not observed. A character to separate *Gloeocystis* from *Coccomyxa* and *Coenocystis* is the presence of the stratified mucilaginous matrix (Nägeli, 1849). The shape of cells and the arrangement of autospores of our alga are very similar to those of *Coccomyxa* Schmidle (1901) and *Coenocystis* Koršikov (1953). Difference between the two genera is the presence or absence of pyrenoid. Since our alga has a pyrenoid in the chloroplast and no stratification in the mucilaginous sheath, it falls into *Coenocystis*. The genus *Coenocystis* currently includes seven species. The main morphological features used in the classification at species rank of *Coenocystis* are summarized in Table 1. According to Komárek and Fott (1983), these species are divided into two groups on the basis of the shape of mature cells, one with spheroidal and the other with ovoidal to ellipsoidal. The former group includes only one species, *Co. tapasteana* Komárek, with a cup-shaped chloroplast. The mature cells of our alga are ellipsoidal to spheroidal in shape, and the chloroplast is cup-shaped often with deeply divided lobes, by which our alga differs from *Co. tapasteana*. The ovoidal to ellipsoidal group of *Coenocystis* is furthermore divided into two subgroups on the basis of the cellular morphology, one being symmetrical and the other asymmetrical. The mature cells of our alga are symmetrical in shape, although the young cells are sometimes asymmetrical. The mature cells of *Co. planctonia*, *Co. subcylindrica* Koršikov and *Co. micrococca* Komárek are symmetrically ovoidal in shape. Of these species, *Co. planctonica* and

Table 1. Comparison of morphological features in species of *Coenocystis*

Species	Shape of mature cells	Size of maure cells (μm)	Symmetry or asymmetry of cells	Number of autospores	Shape of chloroplast	Number of pyrenoid
C. tapasteana	spheroidal	4.8–6	symmetry	(2)–4–8(–16)	cup	1
C. planctnica	widely ovoidal	8–20 ×(8–)10–25	symmetry	4–8	separated	1
C. subcylindrica	widely ovoidal	3.5–8.5 ×5–12	symmetry	(2–)4–8(–16)	trough	1
C. micrococca	ovoid	1.6–2.4	symmetry	2(–4)	cup	1
C. reniformis	ellipsoidal	6–7 ×10–12.8	asymmetry	(2–)4	(not described)	1 or 2
C. asymmetrica	ellipsoidal	2–3.5 ×2.6–4.8	asymmetry	(2–)4	trough, cup	1
C. quadriguloides	ellipsoidal	4–5 ×10–12	asymmetry	4	trickle	1
C. inconstans	ellipsoidal to spheroidal	3.5–6.5 ×5–9.5 3.5–8	symmetry	2–4(–8)	cup often with a few deeply divided lobes	1

Co. subcylindrica form 4–8(–16) autospores in a parent cell, while our alga forms 2–4(–8) autospores. In addition, the size of cells is larger in *Co. planctonica* and *Co. subcylindrica* than in our alga. *Coenocystis micrococca* forms 2–4 autospores, but differs from our alga in considerably smaller size of cells. Our alga is therefore recognized as a new species.

The ultrastructural features of *Coenocystis* inconstans resemble those of *Chlorella mirabilis* observed by Hanagata et al. (unpublished) and of *Parietchloris incisa* (Reisigl) S. Watanabe (Watanabe et al. 1996), especially in having a cell wall lacking a trilaminar structure and pyrenoid traversed by unbranched thylakoid lamellae in almost parallel arrangement. The 18S rDNA tree shows that *Co. inconstans* forms a monophyletic relationship with *Ch. mirabilis*. The main distinction of *P. incisa* from *Co. inconstans* and *Ch. mirabilis* concerns the method of reproduction. In *Co. inconstans* and *Ch. mirabilis* asexual reproduction takes place by the formation of autospores, while in *P. incisa* by the formation of zoospores.

The genus *Coenocystis* has been placed in the Chlorococcales (Komárek and Fott 1983). Recently, the chlorococcalean algae are divided into three groups according to features of their flagellar apparatus. Some algae with directly opposed basal bodies are now placed in the Sphaeropleales (Deason et al. 1991). The algae with counterclockwise orientation of basal bodies are now assigned to the Trebouxiophyceae (Friedl 1995), while the algae with clockwise orientation basal bodies remain in the Chlorococcales. This classification is supported by the phylogenetic relationship (Friedl 1995, Nakayama et al. 1996). The taxonomic position of Coenocystis can not be analyzed on the basis of the ultrastructural features of flagella apparatus, since this genus lacks the flagellate stages. We therefore follow at present the classification of Komárek anf Fott (1983) and tentatively place our new species *Coenocystis inconstans* in the family Radiococcaceae, the Chlorococcales, the Chlorophyceae.

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花方信孝[a]，千原光雄[b]：**樹皮着生緑藻の1新種 *Coenocystis inconstans***

ニュージーランド・クライストチャーチに植栽された *Ulmus campestris* var. *vulgaris* の樹皮より単離した鞭毛をもたない緑藻について分類学的な研究を行った．この藻は単細胞で，細胞は長楕円形から球形であり粘質物に囲まれている．葉緑体は盃状で，時折縁辺部が裂片になる．ピレノイドにはいくつかのチラコイドがほぼ平行に貫通し，デンプン粒はチラコイド間，まれにピレノイドの周囲にも存在している．自生胞子は2–8個形成され，それらを放出した後の親細胞壁は観察されない．これらの形質は基本的に *Coenocystis* 属と一致し，このような特徴をもつ既知種がないので新種と認め，*Coenocystis inconstans* Hanagata & Chihara の種名を用意した．この新種は，18S rDNA領域の塩基配列の解析ではトレボウクシア藻綱に属し，*Chlorella ellipsoidea* および *Chlorella mirabilis* と近縁とする結果を得たが，鞭毛装置の解剖学的所見が得られないこと，および近縁の藻群の分子系統学の知見が充分でないことから，ここではKomárek and Fott (1983) の分類に従い，緑藻綱，クロロコックム目，ラディオコックス科に所属させる扱いとした．

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